



Appl. No. 10/826,812
Amdt. dated August 13, 2007
Reply to Office Action of February 12, 2007

PATENT

Amendments to the Specification:

Please replace the paragraph beginning at page 4, line 4, with the following amended paragraph:

The nucleotide sequences of exemplary natural cDNAs encoding drosophila 1, drosophila 2, C. elegans, human1, human 2 and mouse 1 Robo polypeptides are shown as ~~SEQ ID NOS:1, 3, 5, 7, 9 and 11~~ SEQ ID NOS:1, 3, 5, 7, 9 (and 15 and 16), and 11, respectively, and the full conceptual translates are shown as ~~SEQ ID NOS: 2, 4, 6, 8, 10 and 12~~ SEQ ID NOS: 2, 4, 6, 8, 10 (and 17 and 18), and 12, respectively. The Robo polypeptides of the invention include incomplete translates of ~~SEQ ID NOS:1, 3, 5, 7, 9 and 11~~ SEQ ID NOS:1, 3, 5, 7, 9 (and 15 and 16), and 11 and deletion mutants of ~~SEQ ID NOS: 2, 4, 6, 8, 10 and 12~~ SEQ ID NOS: 2, 4, 6, 8, 10 (and 17 and 18), and 12, which translates and deletion mutants have Robo-specific amino acid sequence, binding specificity or function. Preferred translates/deletion mutants comprise at least a 6, preferably at least an 8, more preferably at least a 32, most preferably at least a 64 residue domain of the translates. In a particular embodiment, the deletion mutants comprise one or more structural/functional Robo immunoglobulin, fibronectin or cytoplasmic motif domains described herein. For example, soluble forms of the disclosed Robo polypeptides which comprise one or more Robo IG domains, and especially fusions of two or more Robo IG domains, particularly fusions of IG#1 and #2, provide competitive inhibitors of Robo-mediated signaling. Exemplary such deletion mutants and recombined deletion mutant fusions include human Robo 1 (SEQ IDNO:8) residues 1-67; 68-167; 168-259; 260-350; 351-451; 1-167; 1-259; 1-350; 1-451; 68-259; 1-67 joined to 168-259; and 1-67 joined to 260-451.

Please replace the paragraph (Table 1 heading) beginning at page 4, line 24, with the following amended paragraph:

Table 1. Sequence Alignment of Robo Family Members: The complete amino acid alignment of the predicted Robo proteins encoded by *drosophila robo 1* (D1, SEQ ID NO: 2) and Human *robo 1* (H1, SEQ ID NO: 8) are shown. The extracellular domain of *C. elegans robo* (CE, ~~SEQ ID~~

~~NO:6~~ SEQ ID NO:13; Sax-3; Zallen et al., 1997), the extracellular domain of *Drosophila robo 2* (D2, ~~SEQ ID NO:4~~ SEQ ID NO:14), and partial sequence of Human *robo 2* (H2, SEQ ID NO:10) are also aligned. The D2 sequence was predicted by the gene-finder program Grail. The position of immunoglobulin domains (~~Ig~~) (IG), fibronectin domains (FN), the transmembrane domain (TM), and conserved cytoplasmic motifs are indicated. The extracellular domain of rat *robo 1* is nearly identical to HI.

Please replace the paragraph (Table 4) at page 11, lines 5-19, with the following:

Table 4. EST:yu23dl1 sequences compared to H-Robol. yu23dl1 refers to the fragment of DNA which was sequenced. The fragment was sequenced from both ends generating the following two sequences: H77734 and H77733. yu23dl1 is an unspliced cDNA. Only bases 59-215 match the coding sequence of H-Robol (502-651). The remaining bases are intronic. No bases of H77733 match the coding sequence of H-Robol.

LRDDFRQNPSDVMVAVGEPAVMECQPPRGHPEPTISWKKDGSPLDDKDER H-Robol (SEQ ID NO:19)
LRDDFRQKPSDVMVAVGEPAVMECQPPRGHPEPTISWKKDGSPLDDKDER EST H77734 (SEQ ID NO:20)

There is an error in the sequence, a T to G change which results in the amino acid N being replaced by K. The sequence is shown below and has been reversed for clarity:

TACTTCGGGATGACTTCAGACAAAAACCTTCGGATGTCATGGTTGCAGTA H-Robol (SEQ ID NO:21)
TACTTCGGGATGACTTCAGACAAAAACCTTCGGATGTCATGGTTGCAGTA EST H77734 (SEQ ID NO:22)
L R D D F R Q K P S D V M V A V (SEQ ID NO:23)

N

Please replace the paragraph (Table 5) beginning at page 11, line 22, with the following:

Table 5. EST:yq76el2 sequences compared to H-Robol. yq76el2 refers to the fragment of DNA which was sequenced. The fragment was sequenced from both ends generating the following two sequences: H52936 and H52937 (the latter has been reversed for clarity). The sequences can be seen to overlap in the middle. A gap indicates a frameshift error. Note that errors only occur in one sequence at any one position.

```
GPLVSDMDTDAPEEEEEDEADMEVAKMQTRRLRLRGLEQTPASSV  H-Robol  (SEQ ID NO:24)
GPLVSDMDTDAPEEEEEDEADMEVAKMQT.RLLLRGLEQTPASSV  EST H52936  (SEQ ID NO:25)

GDLESSVTGSMINGWGSASEEDNISSGRSSVSSSDGSFFTDADF  H-Robol
GDLESSVTGSMINGWGSASEEDNISSGRSSVSSSDGSFFTDADF  EST H52936

AQAVAAA AEYAGLKVARRQMQDA AGR RHFH AS QC PRPT  H-Robol
AQAVAAA AEYAGLKVARRQMQDA AGR RHFH AF QC PRPT  EST H52936
      ?AAT A?YAGLKVARRQMRDA AGR RHFH AS QC PRPT  EST H52937  (SEQ ID NO:26)

SPVSTDSNMSAAVMQKTRPAKKLKHQPGHLRRETYTDDLPPPPV  H-Robol
SPVFTDSNM                                           EST H52936
SPVSTDSNMSAAVMQKTRPAKKLKHQPGHLRRETYTDDLPPPPV  EST H52937
PPPAIKSPTAQSKTQLEVRPVVVPKLPSMDARTDK           H-Robol
PPPAIKSPTAQSKTQLEVRPVVVPKLPSMDARTDK           EST H52937
```

Please replace the paragraph beginning at page 13, line 20, with the following:

In a particular embodiment, the subject polypeptides are used to generate Robo- or human Robo-specific antibodies. For example, the Robo- or human Robo-specific peptides described above are covalently coupled to keyhole limpet antigen (KLH) and the conjugate is emulsified in Freund's complete adjuvant. Laboratory rabbits are immunized according to

conventional protocol and bled. The presence of Robo-specific antibodies is assayed by solid phase immunosorbant assays using immobilized Robo polypeptides of ~~SEQ ID NOS: 2, 4, 6, 8, 10 or 12~~ SEQ ID NOS: 2, 4, 6, 8, 10 (and 17 and 18), or 12. Human Robo-specific antibodies are characterized as uncross-reactive with non-human Robo polypeptides (SEQ ID NOS:2, 4, 6, and 12).

Please replace the paragraph beginning at page 14, line 30, with the following:

The invention also provides nucleic acid hybridization probes (Tables 6, 7) and replication / amplification primers (~~Tables 7, 8~~) (Tables 8, 9) having a Robo cDNA specific sequence comprising ~~SEQ ID NOS:1, 3, 5, 7, 9 or 11~~ SEQ ID NOS:1, 3, 5, 7, 9 (and 15 and 16), or 11 and sufficient to effect specific hybridization thereto (i. e. specifically hybridize with ~~SEQ ID NOS:1, 3, 5, 7, 9 or 11~~ SEQ ID NOS:1, 3, 5, 7, 9 (and 15 and 16), or 11, respectively, in the presence of CDO cDNA.

Please replace the paragraph (Table 5, amended to correct numbering to Table 6) beginning at page 15, line 4, with the following:

~~Table 5~~ Table 6. Hybridisation Probes for Human Roundabout 1

Immunoglobulin Domain #1 (SEQ ID NO:27)

CCACCTCGCATTGTTGAACACCCCTTCAGACCTGATTGTCTCAAAGGAGAACCTGCAACTTTGAACTGCAAAGCT
GAAGGCCGCCCCACACCCACTATTGAATGGTACAAAGGGGGAGAGAGAGTGGAGACAGACAAAGATGACCCTCGC
TCACACCGAATGTTGCTGCCGAGTGGATCTTTATTTTCTTACGTATAGTACATGGACGGAAAAGTAGACCTGAT
GAAGGAGTCTATGTCTGTGTAGCAAGGAATTACCTTGGAGAGGCTGTGAGCCACAATGCATCGCTGGAAGTAGCC
ATA

Immunoglobulin Domain #2 (SEQ ID NO:28)

CTTCGGGATGACTTCAGACAAAACCCCTTCGGATGTCATGGTTGCAGTAGGAGAGCCTGCAGTAATGGAATGCCAA
CCTCCACGAGGCCATCCTGAGCCCACCATTTTCATGGAAGAAAGATGGCTCTCCACTGGATGATAAAGATGAAAGA
ATAACTATACGAGGAGGAAAGCTCATGATCACTTACACCCGTAAAAGTGACGCTGGCAAATATGTTTGTGTTGGT
ACCAATATGGTTGGGGAACGTGAGAGTGAAGTAGCCGAGCTGACTGTCTT

Immunoglobulin Domain #3 (SEQ ID NO:29)

AGAGAGACCATCATTTGTGAAGAGACCCAGTAACCTGGCAGTAACCTGTGGATGACAGTGCAGAATTTAAATGTGA
GGCCCGAGGTGACCCGTGTACCTACGTACGATGGAGGAAAGATGATGGAGAGCTGCCCAAATCCAGATATGAAAT
CCGAGATGATCATACCTTGAAAATTAGGAAGGTGACAGCTGGTGACATGGGTTTCATACACTTGTGTTGCAGAAAA
TATGGTGGGCAAAGCTGAAGCATCTGCTACTCTGACTGTTCAAGAACC

Immunoglobulin Domain #4 (SEQ ID NO:30)

CCACATTTTGTGTGAACCCCGTGACCAGGTTGTTGCTTTGGGACGGACTGTAACTTTTTCAGTGTGAAGCAACC
GGAAATCCTCAACCAGCTATTTTCTGGAGGAGAGAAGGGAGTCAGAATCTACTTTTCTCATATCAACCACCACAG
TCATCCAGCCGATTTTCAGTCTCCAGACTGGCGACCTCACAATTACTAATGTCCAGCGATCTGATGTTGGTTAT
TACATCTGCCAGACTTTAAATGTTGCTGGAAGCATCATCACAAGGCATATTTGGAAGTTACAGATGTGATTGCA

Immunoglobulin Domain #5 (SEQ ID NO:31)

GATCGGCCTCCCCAGTTATTCGACAAGGTCCTGTGAATCAGACTGTAGCCGTGGATGGCACTTTCGTCTCTCAGC
TGTGTGGCCACAGGCAGTCCAGTGCCACCATTCTGTGGAGAAAGGATGGAGTCCTCGTTTCAACCCAAGACTCT
CGAATCAAACAGTTGGAGAATGGAGTACTGCAGATCCGATATGCTAAGCTGGGTGATACTGGTCGGTACACCTGC
ATTGCATCAACCCCCAGTGGTGAAGCAACATGGAGTGCTTACATTGAAGTTCAAGAATTTG

Fibronectin Domain #1 (SEQ ID NO:32)

GAGTTCAGTTCAGCCTCCAAGACCTACTGACCCAAATTTAATCCCTAGTGCCCCATCAAAACCTGAAGTGACAG
ATGTCAGCAGAAATACAGTCACATTATCGTGGCAACCAAATTTGAATTCAGGAGCAACTCCAACATCTTATATTA
TAGAAGCCTTCAGCCATGCATCTGGTAGCAGCTGGCAGACCGTAGCAGAGAATGTGAAAACAGAAACATCTGCCA
TTAAAGGACTCAAACCTAATGCAATTTACCTTTTCTTGTGAGGGCAGCTAATGCATATGGAATTAGTGATC

Fibronectin Domain #2 (SEQ ID NO:33)

CAAGCCAAATATCAGATCCAGTGAAAACACAAGATGTCCTACCAACAAGTCAGGGGGTGGACCACAAGCAGGTCC
AGAGAGAGCTGGGAAATGCTGTTCTGCACCTCCACAACCCACCGTCCTTTCTTCTCTTCCATCGAAGTGCACT
GGACAGTAGATCAACAGTCTCAGTATATACAAGGATATAAAATTCTCTATCGGCCATCTGGAGCCAACACGGAG
AATCAGACTGGTTAGTTTTTTGAAGTGAGGACGCCAGCCAAAAACAGTGTGGTAATCCCTGATCTCAGAAAGGGAG
TCAACTATGAAATTAAGGCTCGCCCTTTTTTTAATGAATTTCAAGGAGCAG

Fibronectin Domain #3 (SEQ ID NO:34)

ATAGTGAATCAAGTTTGCCAAAACCCCTGGAAGAAGCACCCAGTGCCCCACCCCAAGGTGTAAGTGTATCCAAGA
ATGATGGAAACGGAAGTCAATTCTAGTTAGTTGGCAGCCACCTCCAGAAGACACTCAAAATGGAATGGTCCAAG
AGTATAAGGTTTGGTGTCTGGGCAATGAACTCGATACCACATCAACAAAACAGTGGATGGTTCCACCTTTTCCG
TGGTCATTCCTTTTCTTGTTCCTGGAATCCGATACAGTGTGGAAGTGGCAGCCAGCACTGGGGCTGGGTCTGGGG
TAAAG

Transmembrane Domain (SEQ ID NO:35)

AGATTTCAAGTGTGGTGAAGCAGCCGGCCTTCATAGCAGGTATTGGAGCAGCCTGTTGGATCATCCTCATGGTCT
TCAGCATCTGGCTTTATCGACACCG

Cytoplasmic Motif #1 (SEQ ID NO:36)

AATCTGAAGGATGGGCGTTTTGTCAATCCATCAGGGCAGCCTACTCCTTACGCCACCACTCAGCTCATCCAGTCA
AACCTCAGCAACAACATGAACAATG

Cytoplasmic Motif #2 (SEQ ID NO:37)

CCCAAGGTACCAAAACAGGTGGCATGAAGTGGGCAGACCTGCTTCCTCCTCCCCAGCACATCCTCCTCCACAC
AGCAATAGCGAAGAGTACAACATTT

Cytoplasmic Motif #3 (SEQ ID NO:38)

CCAGCCAGGACATCTGCGCAGAGAAACCTACACAGATGATCTTCCACCACCTCCTGTGCCGCCACCTGCTATAAA
GTCACCTACTGCCCAATCCAAGACA

Please replace the paragraph (Table 6, amended to correct numbering to Table 7)
beginning at page 17, line 1, with the following:

~~Table 6~~ Table 7. Hybridisation Probes for Human Roundabout 2

Immunoglobulin Domain #4 (SEQ ID NO:39)

CAGATTGTTGCTCAAGGTGGAACAGTGACATTTCCCTGTGAACTAAAGGAAACCCACAGCCAGCTGTTTTTTGG
CAGAAAGAAGGCAGCCAGAACCTACTTTTCCCAAACCAACCCAGCAGCCCAACAGTAGATGCTCAGTGTACCA
ACTGGAGACCTCACAAATACCAACATTCAACGTTCCGACGCGGGTTACTACATCTGCCAGGCTTTAACTGTGGCA
GGAAGCATTTTAGCAAAAGCTCAACTGGAGGTTACTGATGTTTTGACA

Immunoglobulin Domain #5 (SEQ ID NO:40)

GATAGACCTCCACCTATAATTCTACAAGGCCAGCCAACCAAACGCTGGCAGTGGATGGTACAGCGTTACTGAAA
TGTAAGCCACTGGTGATCCTCTTCCTGTAATTAGCTGGTTAAAGGAGGGATTTACTTTTCCGGGTAGAGATCCA
AGAGCAACAATTCAAGAGCAAGGCACACTGCAGATTAAGAATTTACGGATTTCTGATACTGGCACTTATACTTGT
GTGGCTACAAGTTCAAGTGGAGAGGCTTCCTGGAGTGCAGTGTGGATGTGACAGAGTCT

Fibronectin Domain #1 (SEQ ID NO:41)

GGAGCAACAATCAGTAAAACTATGATTTAAGTGACCTGCCAGGGCCACCATCCAAACCGCAAGTCACTGATGTT
ACTAAGAACAGTGTACCTTGTCTGGCAGCCAGGTACCCCTGGAACCCTTCCAGCAAGTGCATATATCATTGAG
GCTTTCAGCCAATCAGTGAGCAACAGCTGGCAGACCGTGGCAAACCATGTAAAGACCACCCTCTATACTGTAAGA
GGACTGCGGCCCAATACAATCTACTTATTCATGGTCAGAGCGATCAACCCCAAGGTYTCAGTGACCCAAGT

Please replace the paragraph (Table 7, amended to correct numbering to Table 8)
beginning at page 17, line 20, with the following:

~~Table 7~~ Table 8. Primer Pairs for PCR of Human Roundabout 1 Domains

Immunoglobulin Domain #1

Forward: 5' CCACCTCGCATTGTTGAACACCCTTCAGAC 3' (SEQ ID NO:42)

Reverse: 5' ATGGCTACTTCCAGCGATGCATTGTGGCTC 3' (SEQ ID NO:43)

Immunoglobulin Domain #2

Forward: 5' CTTCGGGATGACTTCAGACAAAACCCTTCG 3' (SEQ ID NO:44)

Reverse: 5' TAAGACAGTCAGCTCGGCTACTTCACTCTC 3' (SEQ ID NO:45)

Immunoglobulin Domain #3

Forward: 5' AGAGAGACCATCATTTGTGAAGAGACCCAG 3' (SEQ ID NO:46)

Reverse: 5' AGGTTCTTGAACAGTCAGAGTAGCAGATGC 3' (SEQ ID NO:47)

Immunoglobulin Domain #4

Forward: 5' CCACATTTTGTGTGAAACCCGTGACCAG 3' (SEQ ID NO:48)

Reverse: 5' TGCAATCACATCTGTAACCTCCAAATATGC 3' (SEQ ID NO:49)

Immunoglobulin Domain #5

Forward: 5' ATCGGCCTCCCCAGTTATTCGACAAGGTC 3' (SEQ ID NO:50)

Reverse: 5' CAAATTCCTGAACTTCAATGTAAGCACTCC 3' (SEQ ID NO:51)

Fibronectin Domain #1

Forward: 5' GAGTTCCAGTTCAGCCTCCAAGACCTACTG 3' (SEQ ID NO:52)

Reverse: 5' TCACTAATTCCATATGCATTAGCTGCCCTC 3' (SEQ ID NO:53)

Fibronectin Domain #2

Forward: 5' CAAGCCAAATATCAGATCCAGTGAAAACAC 3' (SEQ ID NO:54)

Reverse: 5' ATCTGCTCCTTGAAATTCATTAAAAAAGG 3' (SEQ ID NO:55)

Fibronectin Domain #3

Forward: 5' ATAGTGAAATCAAGTTTGCCAAAACCCTG 3' (SEQ ID NO:56)

Reverse: 5' CTCTTTACCCAGACCCAGCCCCAGTGCTG 3' (SEQ ID NO:57)

Transmembrane Domain

Forward: 5' GGACCAAGTCAGCCTCGCTCAGCAGATTTTC 3' (SEQ ID NO:58)

Reverse: 5' ACTAGTAAGTCCGTTTCTCTTCTTGCGGTG 3' (SEQ ID NO:59)

Cytoplasmic Motif #1

Forward: 5' CTGAAGGATGGGCGTTTTGTCAATCCATC 3' (SEQ ID NO:60)

Reverse: 5' GTCCAGTGGTTTCCAGTGCTTCTCGCCAG 3' (SEQ ID NO:61)

Cytoplasmic Motif #2

Forward: 5' GGCACAAGAAAGGGGCAAGAACACCCAAGG 3' (SEQ ID NO:62)

Reverse: 5' ATAGCTTTCATCTACAGAAATGTTGTACTC 3' (SEQ ID NO:63)

Cytoplasmic Motif #3

Forward: 5' ACCAGACCAGCCAAGAACTGAAACACCAG 3' (SEQ ID NO:64)

Reverse: 5' GTACTTCCAGCTGTGTCTTGGATTGGGCAG 3' (SEQ ID NO:65)

Please replace the paragraph (Table 8, amended to correct numbering to Table 9) beginning at page 18, line 34, with the following:

~~Table 8~~ Table 9. Human Roundabout 2 Primer Pairs

Immunoglobulin Domain #4

Forward: 5' GTTGCTCAAGGTCGAACAGTGACATTTCCC 3' (SEQ ID NO:66)

Reverse: 5' TGTCAAAACATCAGTAACCTCCAGTTGAGC 3' (SEQ ID NO:67)

Immunoglobulin Domain #5

Forward: 5' GATAGACCTCCACCTATAATTCTACAAGGC 3' (SEQ ID NO:68)

Reverse: 5' GACTCTGTACATCCAGCACTGCACTCCAG 3' (SEQ ID NO:69)

Fibronectin Domain #1

Forward: 5' CAATCAGTAAAACTATGATTTAAGTG 3' (SEQ ID NO:70)

Reverse: 5' TCGCTCTGACCATGAATAAGTAGATTG 3' (SEQ ID NO:71)

Please replace the paragraph beginning at page 19, line 22, with the following:

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i. e. unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. The subject recombinant nucleic acids comprising the nucleotide sequence of ~~SEQ ID NOS:1, 3, 5, 7, 9 or 11~~ SEQ ID NOS:1, 3, 5, 7, 9 (and 15 and 16), or 11, or fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i. e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, more

preferably fewer than 500 bp, which is at a terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

Please replace the paragraph (Table 18) beginning at page 28, line 11, with the following:

Table 18. Conserved Cytoplasmic Motifs: Amino acid alignments of the three conserved cytoplasmic motifs are shown below the structure; in *C. elegans robo*, motifs #2 and #3 have been switched to provide a better alignment.

Conserved Cytoplasmic Motif #1

PDNPTPYATTMIIGTSS	1050	Drosophila roundabout-I	(SEQ ID NO:72)
SGQPTPYATTQLIQSNL	1083	Human roundabout-I	(SEQ ID NO:73)
NASPAPYATSSILSPHQ	1088	Drosophila roundabout-II	(SEQ ID NO:74)
HDDPSPYATTTLVLSNQ	1049	<i>C. elegans</i> roundabout	(SEQ ID NO:75)
PtPYATT.hh....		Consensus (where h is I, L or V)	(SEQ ID NO:76)

Conserved Cytoplasmic Motif #2

INWSE.FLPPPPEHPPPSSTYG.Y	1119	Drosophila roundabout-I	(SEQ ID NO:77)
MNWAD.LLPPPPAHPPPHSNSEY	1202	Human roundabout-I	(SEQ ID NO:78)
STWANVPLPPPPVQPLPGTELEHY	31	Human roundabout-II	(SEQ ID NO:79)
KTLM.D.FIPPPPSNPPPP.GGHVY	1168	<i>C. elegans</i> roundabout-I	(SEQ ID NO:80)
nW...hhPPPP. PPP.s....Y		Consensus (where h is hydrophobic)	(SEQ ID NO:81)

Conserved Cytoplasmic Motif #3

PSPMQPPPPVPVPEGW.Y	1273	Drosophila roundabout-I	(SEQ ID NO:82)
YTDDLPPPPVPPPAIKSP	1493	Human roundabout-I	(SEQ ID NO:83)

YADDLPPPPVPPPAIKSP 90 Mouse roundabout-I (SEQ ID NO:84)
RAPAMPTNPVPPEPPARY 1077 C. elegans roundabout (SEQ ID NO:85)
.....PPPPVPPP..... Consensus (SEQ ID NO:86)

The consensus for the first motif is PtPYATTxhh (SEQ ID NO:76), where x is any amino acid and h is I, L, or V. The presence of a tyrosine in the center of the motif indicates a site for phosphorylation. The other two motifs consist of runs of prolines separated by one or two amino acids and are reminiscent of binding sites for SH3 domains. In particular, the LPPP (SEQ ID NO:87) sequence in motif #2 provides a good binding site for the Drosophila Enabled protein or its mammalian homologue Mena (Niebuhr et al., 1997). All three of these conserved sites can function as binding sites for domains (e.g. SH3 domains) of linker/adaptor proteins functioning in Robo-mediated signal transduction.

Please replace the paragraph beginning at page 33, line 23, with the following:

Identification of Molecular Defects In *robo* Alleles. Southern blots of *robo* alleles and their parental chromosomes were hybridized with fragments from the genomic cosmid clone 106-1435 or partial cDNA clones to identify restriction fragment length polymorphisms affecting the *robo* transcription unit. DNA was obtained from homozygous mutant embryos. 35 cycles of the PCR was subsequently performed on the DNA obtained from half an embryo. Primers specific for the region flanking the CfoI polymorphism used were: ROBO6 (5'-GCATTGGGTCATCTGTAGAG-3'; SEQ ID NO:88) and ROBO23 (5'-AGCTATCTGGAGGGAGGCAT-3'; SEQ ID NO:89). The PCR products were purified on a Pharmacia H300 spin column and sequenced directly.

Please replace the paragraph beginning at page 34, line 4, with the following:

PCR amplification of the D-robo ORF using the primers (5'-GAGTGGTGAATTCAACAGCACCAAACCACAAAATGCATCCC-3'; SEQ ID NO:90) and

(5'-CGGGGAGTCTAGAACACTTCATCCTTAGGTG-3'; SEQ ID NO:91) produced a PCR product with an altered ribosome binding site that more closely matches the *Drosophila* consensus (Cavener, 1987), and has only 21bp of 5' UTR and no 3' UTR sequences. The PCR product was digested with EcoRI and XbaI and cloned into pBluescript (Stratagene) and subsequently, pUAST (Brand and Perrimon 1993). Transformant lines were crossed to *elav-GAL4* and *sca-GAL4* lines which express GAL4 in all neurons, or *ftzng-GAL4* which expresses in a subset of CNS neurons (Lin et al, 1994). Embryos were assayed by staining with MAbs BP102,1D4 and 13C9. For ectopic expression in the *robo* mutant background, the stocks *robo*³ and *robo*⁵ (both protein nulls) were used. Crosses utilized the stocks *w; robo/CyO; UAS-robo* and *w; robo/CyO; elav-GAL4*. Due to the difficulty of maintaining a balanced stock, *robo/+; ftz-ngGAL4/+* males were generated as required.

Please replace the paragraph beginning at page 34, line 17, with the following:

Generation of Fusion Proteins and Antibodies. A six histidine (SEQ ID NO:92) tagged fusion protein was constructed by cloning amino acids 404-725 of the D-robo protein into the PstI site of the pQE31 vector (Qiagen). Fusion proteins were purified under denaturing conditions and subsequently dialyzed against PBS. Immunization of mice and MAb production followed standard protocols (Patel, 1994).

Please cancel the present "SEQUENCE LISTING", pages 1-26, submitted on October 15, 2004, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 55, at the end of the application.

Amendments to the Claims:

This listing of claims will replace all prior listings of claims in the application:

Listing of Claims:

- 1.-9. (cancelled)
10. (currently amended) An isolated ~~Rebo-specific~~ antibody that specifically binds to a polypeptide ~~having at least 95% identity to~~ of SEQ ID NO:8.
11. (previously presented) The antibody of claim 10, wherein the antibody is a polyclonal antibody.
12. (previously presented) The antibody of claim 10, wherein the antibody is a monoclonal antibody.
13. cancelled
14. (previously presented) The antibody of claim 10, wherein the antibody binds to an extracellular domain of the polypeptide.
15. cancelled.
16. (currently amended) The antibody of claim 10, wherein the antibody is ~~raised against a protein comprising~~ binds to a domain selected from the group consisting of:
 - (a) residues 68-167 of SEQ ID NO:8;
 - (b) residues 168-258 of SEQ ID NO:8;
 - (c) residues 259-350 of SEQ ID NO:8;

- (d) residues 351-450 of SEQ ID NO:8;
- (e) residues 451-546 of SEQ ID NO:8;
- (f) residues 547-644 of SEQ ID NO:8;
- (g) residues 645-761 of SEQ ID NO:8; and
- (h) residues 762-862 of SEQ ID NO:8.

17. (currently amended) The antibody of claim 10, wherein the antibody is ~~raised against a protein comprising~~ binds to a fragment of SEQ ID NO:8 selected from the group consisting of residues 18-28, 31-40, 45-65, 106-116, 137-145, 174-184, 214-230, 274-286, 314-324, 399-412, 496-507, 548-565, 599-611, 660-671, 717-730, 780-791, 835-847, 877-891, 930-942, 981-998, 1040-1051, 1080-1090, 1154-1168, 1215-1231, [[.]] 1278-1302, 1378-1400, 1460-1469, 1497-1519, 1606-1626 and 1639-1651.

18. (cancelled)

19. (previously presented) The antibody of claim 10, where the antibody is labeled.

20.-28. (cancelled)